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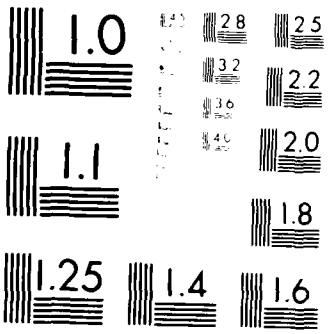

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Model ion channels such as gramicidin, melittin and alamethicin exhibit functional similarities to physiological channels, including voltage-gating, selectivities, activation and inactivation. Since the structural studies of physiological channels are extremely difficult, the structrue-function relations revealed in model channels may provide valuable insights into the physical basis of biological sensory. A great deal of functional (electrical) properties of model channels is known, but with the exception of gramicidin (which unfortunately is not a voltage-gated channel) little progress has been made in determining the structrues of the channels in membrane. We have developed a technique of preparing defect-free lipid multibilayers with ion channels embedded in them, so that spectroscopic and scattering measurements of aligned ion channels in electric field can be made. CD studies of alamethicin channels showed the important orientation effect of -helical peptides on CD and conformation changes of the channel with the sample conditions.

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Introduction

Ion channels are macromolecular pores in membranes that produce and transmit electric signals in living cells. The invention of the patch-clamp technique has made discrete current steps of single ion channels observable. However, partly due to the difficulties in crystallizing membrane proteins in their native forms, in no case is the molecular structure of such a channel or the molecular mechanism of voltage-gating known. One approach to resolving this problem has been the study of model ion channels which are smaller and simpler than physiological channels but exhibit functional similarities. It is reasonable to expect that the structure-function relations of various model channels could be understood in detail to provide insights into the mechanisms by which voltage-gated channels in biological membranes operate. Indeed since the 1960's the interplay between studies of excitable membrane and studies of model pores has greatly sharpened our understanding of the ion transport mechanism(e.g. Hille,1984). Gramicidin, melittin and alamethicin probably represent three different molecular mechanisms of ion channels. Gramicidin (15-amino acid peptide) forms a head-to-head dimeric channel which exhibits gating and ion selectivity; it is so far the best characterized channel. Next in complexity is the melittin (26-amino acid peptide) channel--a tetramer whose structure is only beginning to be understood. Interestingly, at this level of molecular organization, the melittin channel already exhibits voltage-gating, activation and inactivation, the essential features of axon channels (Tosteson and Tosteson, 1984). Alamethicin, a 20-amino-acid peptide, forms oligomeric channels--the number of monomers per channel varies with the sample condition. Consequently the voltage-dependent channel formation of alamethicin is more complicated than melittin. Because the monomers of these model channels are relatively simple, theoretical ideas of channel processes can be built on structural bases. And more importantly, these ideas can be tested experimentally by manipulating the model channels through a wide range of measurement conditions.

So far the experiments on model ion channels were primarily performed with two types of samples. The channels mediated ion conductions were usually measured by using black lipid membranes, whereas the spectroscopic studies such as nuclear magnetic resonance, Raman, dielectric relaxation, circular dichroism and infrared absorption were mostly performed with micellar or vesicular suspensions. It was desirable but difficult to perform spectroscopic or scattering experiments on ion channels under the influence of membrane potentials. This situation is now improved by the defect-free multibilayer samples. As will be described in the following, we have developed a technique of preparing a bulk phase of model ion channels embedded in monodomain, aligned multibilayers of phospholipid between two thin electrodes (tin oxide coatings on silica plates). We are able to apply an electric field across the multibilayers and simultaneously perform spectroscopic or scattering measurements on the embedded ion channels.

Objectives

Our current efforts are concentrated on the circular dichroism (CD) and small-angle neutron scattering (SANS) studies of the alamethicin and melittin channels.

1) CD studies: Peptides incorporated in membrane tend to be α-helical. Ultraviolet CD can be used to study the orientation of the α-helical sections of the monomers with respect to the plane of the membrane. We have already detected changes of the monomer orientation in alamethicin channels with the water content and temperature.

2) SANS studies: With a defect-free multibilayers sample, the lipid contribution to the SANS spectrum can be suppressed (see below). Therefore we expect the scattering by the proteins embedded in the multibilayers to show up clearly. If we can successfully measure the sizes of model ion channels and the 2-dimensional channel-channel correlations in membrane with defect-free multibilayers, it will open a very useful new method for studying membrane proteins.

Our specific objective for the near future is to use CD and SANS to gain structural information on the alamethicin and melittin channels by varying the electric field, lipid, water content and temperature.

Progress Report

1) Defect-free multibilayers

They are smectic lyotrpic liquid crystals consisting of lipid bilayers intercalated with water (about 20% by weight) and with ion channels embedded in bilayers. The technique of preparing defect-free multibilayers samples was published in Huang and Olah (1987) with gramicidin embedded in them. We have since successfully embedded alamethicin and metittin in the multibilayers. The thicknesses of our defect-free samples are a few μm to 100 μm with an area of 40mm^2 to 1 cm^2. The peptide/lipid molar ratio can be from 1/20 to zero. The alignment and the smectic defects are inspected by conoscopy and polarized microscopy. The incorporation of peptides in bilayers is monitored by CD.

2) Preliminary SANS experiment

In a preliminary experiment a defect-free pure lipid multilayers sample, with the neutron beam incident perpendicular to the layers and consequently the scattering momentum transfer $\vec{k}$ in the plane of the layers, gave a small, smooth SANS spectrum. This is contrary to typical multilayers prepared by depositing and drying a vesicular solution on a smooth surface; in such preparations, the multilayers contain disclinations which give a strong peak in the SANS at a $|\vec{k}|$ corresponding to the spacing of bilayers. With the lipid contribution suppressed, we expect the scattering by the proteins embedded in the multibilayers to show up clearly.

3) The orientation effect of α-helical peptides on CD

Although the Moffitt theory about this effect has been assumed and used in the literature, no rigorous experimental proof has been given (mostly because the orientation of α-helices could not be independently checked). We have measured the CD of embedded alamethicin in defect-free multibilayers with the sample tilted at various angles to obtain the rigorous proof of the Moffitt theory. By this experiment we have also

established a rigorous procedure for detecting the alignment of α-helical proteins by CD.

4) Conformation changes of alamethicin channels with hydration and temperature

We have detected this effect with CD. The work is in progress. We believe that we will be able test several proposed models.

5) Effect of membrane thickness on gramicidin channel lifetime--theory

This effect was successfully explained by considering a complete deformation free energy of membrane (Huang, 1986). One of the important consequences of this investigation is the discovery of the shape of membrane deformation when a protein of hydrophobic length shorter than the membrane thickness is incorporated in the membrane. Contrary to the common belief that membrane would dimple, the portion of membrane neighboring the protein is actually flat. As a result, the deformation free energy and its restoring force are both very large (see details in Huang,1986), which are necessary to explain the data on the gramicidin channel lifetime.

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